THE EFFECT OF ULTRA-VIOLET LIGHT RAYS UPON THE DEVELOPMENT OF THE FROG'S EGG.

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II. THE ARTIFICIAL PRODUCTION OF FOLDED (U-SHAPED) EMBRYOS.

This paper is the second of a series dealing with an investigation of the nature and mechanism of causation of certain abnormal, developmental results obtained by raying certain restricted areas of the fertilized ovum of the frog by means of ultra-violet light rays. Since the first paper dealt with the production of a single type-defect, spina bifida, the possibility of production of another constant and fixed experimental result, a folded or U-shaped embryo, justifies the separate consideration of that result in this contribution. Once again the value of the physical method of attack upon the complex problem of developmental reaction is demonstrated. In this problem we are concerned with a readily controllable and constant, causative agent on the one hand and a uniform embryological defect on the other. This one technical condition permits of a more thorough analytical inquiry into the mechanism of production of this developmental defect. In addition to this consideration, however, as is to be expected the experiment gives further insight into the genetic constitution of the fertilized, undivided ovum, and, more specifically, helps in the identification and location of the organ forming substances and anlagen. The apparatus used for the experiment was that detailed in the preceding paper. Some of the work was done, however, with an apparatus for the use of which I am indebted to Mr. W. S. Andrews, of the General Electric Company at Schenectady. With both forms of apparatus, the surface area concentration of the rays was increased through the use of convex quartz lenses.

The eggs used were those of the common species of frogs found

around Ithaca and also Schenectady, N. Y. These were collected early in the morning, as soon as possible after laying. They were brought into the laboratory, divested of their jelly, and rayed while still in the undivided or two-celled stage. A thin tin-foil diaphragm perforated by a hole three tenths of a millimeter in diameter shielded most of the surface area of the eggs from the rays but permitted a relatively small portion of that area to be influenced. After raying, the eggs were transferred to a jar containing 1,000 c.c. of tap water in which they were permitted to develop, the water being changed frequently.

Physicists have definitely established the fact that ultra-violet light rays possess a very slight penetration capacity. We are justified, hence, in assuming that the chemical alterations produced by the rays acting under these conditions were restricted to a superficial layer of protoplasm of the egg in the restricted area mentioned. The interpretations presented in this paper are made, accordingly, upon that basis.

The surface area of the egg subjected to examination in this experiment is best demonstrated by a reference to Fig. 1. A

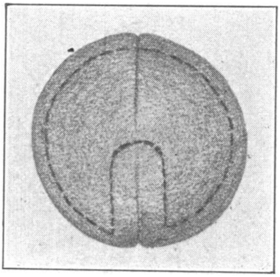

FIG. 1.

two-cell ovum is shown with the animal pole uppermost. The broken line marks out the area investigated. As is to be seen this extends from the region of the equator up towards the animal pole but is exclusive both of the pole itself and of a narrow median strip which connects the latter with the equator. As can be seen this region is exclusive of the gray crescent. Different portions of this area were rayed successively with the three tenths millimeter beam of light. As a result, when development

was permitted to progress without further interference, the long axis of the trunk of the embryos was invariably bent towards one or the other side. The level of this bending or flexure of the trunk depended directly upon the location of the area influenced by the light.

Since the jelly which envelops the eggs had been found in the spina bifida experiments to be impervious to ultra-violet light rays, care was taken to remove it prior to the experiments. As a result a marked increase in the percentage of positive results followed the raying. Where, as was the case more particularly with green frog's eggs, the jelly could be removed almost completely, about every egg rayed developed an abnormality which conformed to this general, U-shaped type. Three type-experiments may be presented for the purpose of demonstrating the constancy of results. On May 27, 1914, 9 eggs in the two-celled stage were rayed, each for 1 minute. One died before development had progressed more than 24 hours. Of the 8 remaining all were U-shaped. Three of these were permitted to develop until the tail and head were well formed. The others were killed at earlier developmental stages. On June 18, 1914, 25 eggs were rayed, each for 30 seconds. These were in the one- and two-celled stage. Various portions of the prescribed area above the equator were influenced. Three died within 24 hours, one lived until the neural groove had disappeared and presented a marked curvature of the trunk. All of the remaining 21 were U-shaped. On June 23, 1914, an exposure of 19 eggs in the one- and two-celled stage was made, each for 30 seconds. Eight of these were rayed in the designated area, and 11 along the equator. One of the eight died early, of the remaining 7 all were U-shaped. The 11 others demonstrated spina bifida.

The inference seems justifiable, therefore, that the constant type of defect produced is the result of a constant and uniform degree of alteration of the superficially-placed egg substance in the area illuminated. Just what the nature of this effect is we are at present unable to state. It would appear that the protoplasm, using this term in its broadest sense, had been modified in some chemical way to a degree which not only rendered it unfit for participation in the subsequent chemical ontogenetic

processes of which it normally was a part but, in addition, caused its presence to act as a mechanical hindrance to the developmental shifting of anlagen.

Studies of the action of ultra-violet light rays have produced proof that proteins, carbohydrates, and lipins may be chemically altered by means of this form of energy. To review the experimental results briefly: Massol and Kluyver have ascertained that starches may be altered, and Stoklasa, Zdobnický, Chauchard and Mazoné, and Pougnet that they may be ultimately broken up. Diastase may be liberated in plants (Maquenne and Demoussy). Bierry, Henri, and Ranc have inverted cane sugar. Agulhon, Maurain and Warcollier, and Raybaud presented evidence that the action of enzymes in the presence of oxygen was weakened and destroyed by ultra-violet light. Furthermore, albumen may be coagulated (Bovie) and the iodine content of fats may be lowered (Roemer and Sames). Thus we may reasonably infer that ultra-violet light rays are capable of bringing about certain chemical modifications of the protoplasm of the cell to all or to part of which we may attribute the abnormality in development noted. The evidence is lacking at present, however, which might enable us to associate this developmental result with a specific alteration of protein, of carbohydrate, or of lipin.

It was pointed out in the preceding paper that the superficial mass of altered protoplasm situated between the approximating neural ridges prevented their normal fusion with the consequent formation of a complete neural tube. The condition of spina bifida resulted, therefore, as a direct result of the mere mechanical interruption of this process. The action of the altered protoplasm was owing entirely to its passive function as a mass which mechanically interfered with the medianward, migratory movement of the hemineural anlagen. At least, in this one factor could be found sufficient evidence as the causative condition of this abnormality.

In the present instance of the U-shaped embryo, however, the inert mass was located lateral to and above the level of the hemineural anlagen. It could not, therefore, by virtue of interposition mechanically interfere with the approximation of the

neural tube-halves. Because of its lateral position and close relationship to one of these halves it might exert, however, a restraining influence upon the rate of migration of the tube-half of the corresponding side of the egg. This retardation of the shifting of the hemineural anlagen on the affected side induced a correspondingly exaggerated migratory process in the anlagen upon the unaffected side of the egg. As a result of this loss of coördinated, migratory movement, the anlagen upon the affected side had not yet reached the median plane before fusion took place with the unaffected anlagen by reason of their exaggerated migration. The normal angularity of the hemineural long-axis persisted more or less completely, therefore, in the adult tadpole as a result of this process. This was expressed by the curvature of the trunk invariably towards the affected side. The location of this flexure, as was stated above, was directly dependent upon the segmental level of the affected area of protoplasm.

It is a singular fact that the histological sections failed to demonstrate a single gross or microscopic defect in these embryos. The viscera developed normal in shape and in size. No defects could be recognized in the muscle segments. The mass of these in the concavity of the flexure was estimated roughly to be the same as that of the corresponding segments on the convex side of the body though their shape was necessarily distorted to fit the concavity. The yolk mass of the trunk of the embryo was completely utilized in the differentiation of the tissues and in the metabolical processes of the embryo. There was no evidence at any time that a portion of this yolk mass had been rendered completely inert by the rays and consequently excluded from participation in the normal chemical developmental processes of the body.

The action of the rays inferentially, therefore, had not induced such chemical changes as would permanently eliminate the substances affected from participation in the normal organo-genetic or somatogenetic processes. But the rate of chemical modification of these substances in participation in the normal metabolical processes of the embryo was, however, retarded. We may find in this retardation of chemical participation additional evidence for the causation of the check of the normal lengthening process

of the embryo on the affected side. The opposite side progressed approximately at its normal, but comparatively greater, rate. As a result, the flexure of the neural tube increased proportionate to the developmental progress of the embryo. In other words, as an embryo increased in age the angle formed by the trunk with the tail became more and more acute until a condition was arrived at finally in which the long axis of the trunk lay parallel to that of the tail.

The sketches presented with this paper are intended merely to demonstrate the general morphological and histological features which are characteristic of the development of these abnormal, U-shaped embryos. It may be stated, however, that no great attempt has been made to make them faithfully representative of all of the histological details of the tadpoles. In many instances the drawings are composite. In every instance, however, the basis of each drawing has been an actual specimen. The figures are to be interpreted, therefore, as merely type representatives of the numerous examples which are illustrative of the peculiarities of this form of abnormal development.

Fig. 2 represents an embryo in the early stages of development

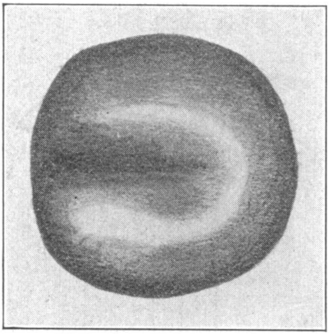

FIG. 2.

viewed from the dorsal aspect. The deep furrow which penetrates from the left, to and beyond the center of the embryo mass represents the line of apposition of the two portions of the right flank of the embryo which is so bent upon itself that the right surface of the tail region is in contact with the right surface

of the head region. Actual continuity of the tissues, however, does not exist. The cephalic end of the embryo, which is represented below and to the left of the sketch, is distinguished by its greater transverse diameter. The area of protoplasm in this specimen affected by the rays lay in the concave bend of the trunk at the extremity of the cleft. It will be noted that this flexure is sharply restricted to a relatively small portion of the embryo. Cephalic to and caudal to this level, both the head-end and the tail-end of the embryo are, respectively, of normal shape.

Occasionally, the two neural tube-halves fail to unite in the caudal region, bringing about a condition which is demonstrated well by Fig. 3. In this the yolk mass is represented as projecting

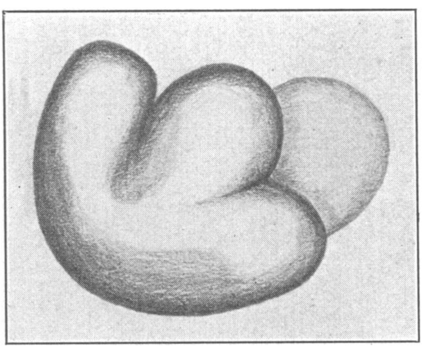

FIG. 3.

to the right between the caudal extremities of the divided neural tube. This is an early developmental feature in the production of the condition of spina bifida, the divided condition of the neural tube-halves persisting in the adult free-swimming tadpole. The bend in this particular embryo is represented as occurring high up in the trunk region. The cephalic end of the embryo is shown with its right surface resting against the right neural tube-half caudally. As was noted in connection with the preceeding figure, so is it likewise true of this, both cephalic to and caudal to the angle of the fold, aside from the features of spina bifida, the appearance of the embryo is practically normal. The stages of development represented by these two figures are relatively early, consequently but little of the ordinary features of external configuration of the embryo can be made out.

A still later stage of development demonstrating a more marked differentiation of the tail from the head region is shown by Fig. 4, while the tadpole in Fig. 5 represents the external features of these tadpoles during the free-swimming period. In this latter picture, the sucker, the eyes, the external gills and the

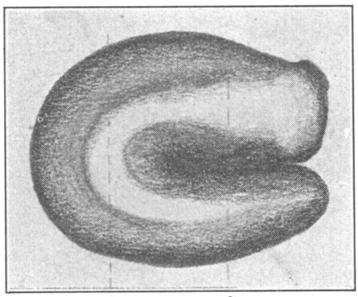

FIG. 4.

tail fins are readily made out. The sharpness of the fold in the trunk in both instances indicates a restricted localization of the alteration produced in the protoplasm of the developing ovum.

These creatures are unable to straighten out the flexure of the trunk. They swim, as was noted before, by means of ordinary tail movements, the vigor of which is not impaired

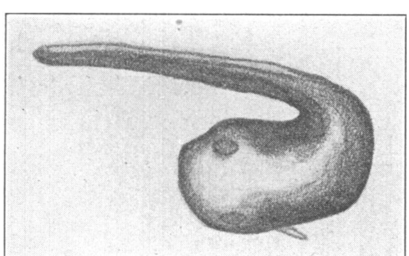

FIG. 5.

in any single instance. As can readily be understood by reference to the figure, however, the abnormal position of the caudal long-axis, with regard to the long-axis of the head and remaining portion of the trunk, is such that the swimming movements of the tail tend to force the embryo about in a circular or spiral

direction. Because of the condition of fixity of the bend progression directly forward is necessarily out of the question.

The deformity represented by Fig. 6 is illustrative of the second mechanism of production of the folded embryos. The concave side of the neural tube, to the right in the figure, is occupied by irregular bulgings of the epidermal surface of the embryo. When the development of embryos conforming to this type is followed from day to day, it is noted that the flexure of the neural tube becomes greater from day to day. The apparent occasion

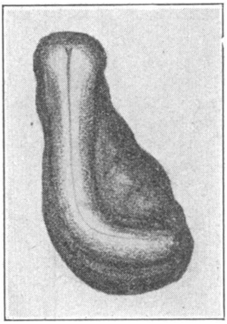

FIG. 6.

of this is referable chiefly to two factors; one, the normal but relatively more rapid growth of the normal myomeres to the left of the longitudinal axis of the body, and the other, to the relatively, and actually slower, growth of them upon the concave side of the median body plane. Histological studies demonstrated as well that differentiation in the myomeres upon the affected side was somewhat retarded.

There are, therefore, these two general developmental mechanisms either of which leads to the production of a U-shaped embryo. In one the angularity of the bend is constant from the time of its first appearance in the embryo, whereas in the other this angularity increases with the succeeding stages of differentiation and growth of the anlagen. The end result is identical, however, in both instances both from the morphological and the histological standpoint. Furthermore, in both instances the segmental relationship of the bend to the area of protoplasm altered in the ovum is the same.

The broken lines shown in Fig. 4 bear numerals which correspond to the planes of section represented by the succeeding figures, 7 and 8. The cross-section represented by 7, therefore, passes through the embryo at the level of the fold in the neural tube. The two halves of the yolk mass with sections of the

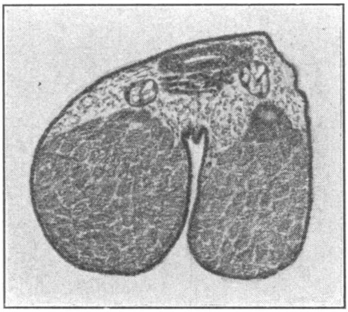

FIG. 7.

enteron which belong, the one to the cephalic end and the other to the caudal end of the embryo, are shown separated from each other by a deep cleft lined by ectoderm. Dorsal to the enteron lie two sections of the notochord with the myomeres sectioned in their long-axis between them. Between and dorsal to these

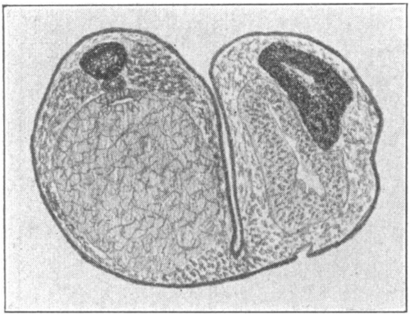

FIG. 8.

two portions of the notochord, the oblique section of the bend in the neural tube, demonstrating the neurocele as well, is to be seen. The level represented by Fig. 8 includes the region of the brain and subjacent pharynx to the right and the trunk to the

left. Both portions of the embryo are connected with each other by a narrow strip of mesenchymal tissue at the bottom of the epidermal cleft. The notochord is to be seen only in the left half of the embryo with the myoblastic tissue to either side of it, the enteron ventral and the neural tube dorsal. The stage is so early that most of the yolk cells are in an undifferentiated condition.

Figs. 9, 10 and 11 are sections of one and the same embryo.

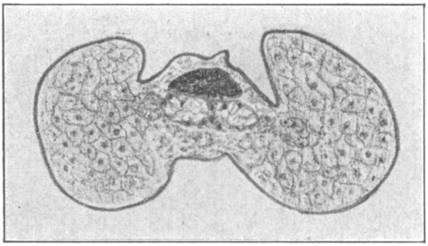

FIG. 9.

In this the bend of the trunk occurred at about the level of its middle segment. The section-knife encountered both the neural tube and the notochord at their curvature. The undifferentiated yolk-mass of the two limbs of the U are connected by a relatively slender commissure-like mass of yolk cells and of

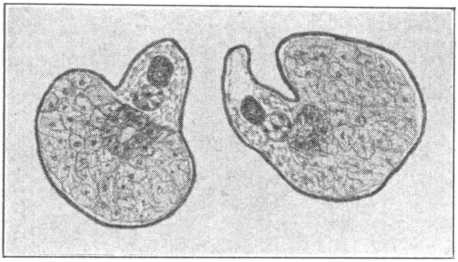

FIG. 10.

mesenchymal tissue. The section of Fig. 10 taken at about the level 8 of Fig. 4 demonstrates on the left the transsected notochord with the cephalic end of the definitive spinal cord corsal to it, both imbedded in myoblastic cells not yet well differentiated. A section of notochord and of spinal cord with the

enteron ventrally placed to them are to be seen in the right half of the sketch. There is no indication at this early stage of a beginning formation of the anlagen either of the liver or of the pancreas. The section represented by 11, however, passes through the head region at the level of the eyes. To the left in this sketch there can be identified the cephalic portion of the neural tube with its well-differentiated walls showing a degree of stratification. The neurocele appears clear and contains no cell

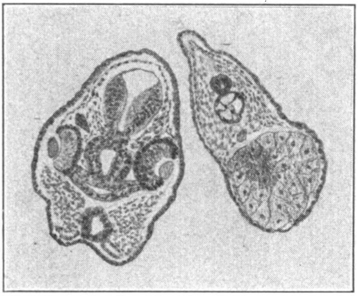

FIG. 11.

detritus. The neuroblasts do not demonstrate any pathological features. A thin layer of mesenchymal tissue intervenes between the roof of the neurocele and the epidermis. Immediately ventral to this section of the brain cavity the knife has encountered another portion of the normally folded brain stem with its ventricle and, projecting to either side from its ventral aspect, a portion of the optic commissure and stalks proceeding laterally to the optic cups. The cytological appearance of these structures presents no departures from normal. The cavities are free from cell detritus. The lens of the eye on both sides is in its normal position. Stratification of the walls of the optic cup, of the stalk and of the brain vesicle have progressed to their normal relative degree. Immediately ventral to these structures in the median line is shown a section through the pharynx, the cells of which, as well, show none but normal features. The right half of the sketch demonstrates the prominent caudal fin, transected neural tube and notochord, and the caudal extremity of the enteron, the last embedded in the dorsal region of the yolk

mass. The mesenchymal tissue to either side of the neural tube and notochord presents normal features.

Comparable to the levels represented by Figs. 7 and 9 is the section represented by Fig. 12. Here again the flexure of the neural tube lies dorsal to the notochord sections. Immediately

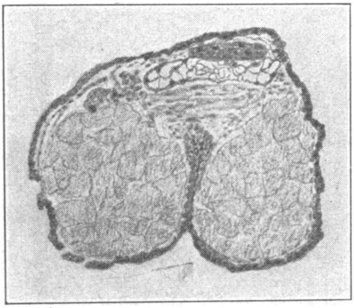

FIG. 12.

ventral to the latter the forms of the elongated myoblasts are to be made out. A relatively early stage of development of the enteron is evidenced by the condition of the yolk cells, the symmetrical masses of which are separated by the deep epidermal cleft.

In the succeeding four figures, 13, 14, 15 and 16, embryos are

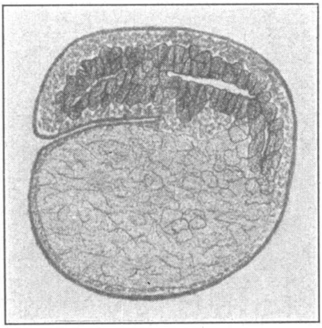

FIG. 13.

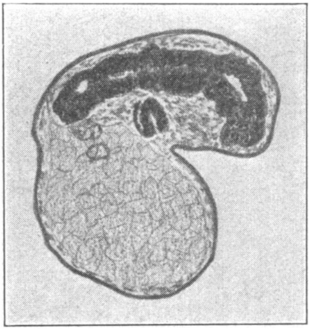

FIG. 14.

represented in each of which the angularity of the flexure was not sufficient to bring the long axis of the two body portions parallel to each other. In each instance, the cephalic extremity

of the embryo projected from the trunk at approximately a right angle. Consequently the neural tube is sectioned in the head region parallel to its long axis, but in the trunk region at right angles to its long axis. In Fig. 14, for instance, the head end of the embryo projects to the right. The walls of the brain

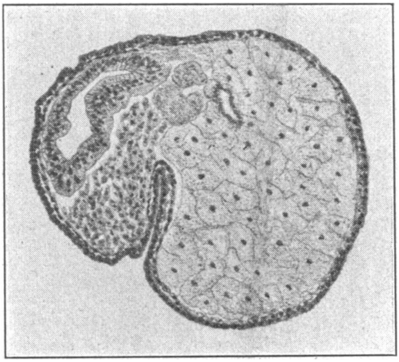

FIG. 15.

vesicle demonstrate no abnormal features. Because of a certain amount of twisting of this extremity but little of the pharynx appears in the section upon the ventral aspect of the brain stem. At the left extremity of the neural tube an almost perfect tran-

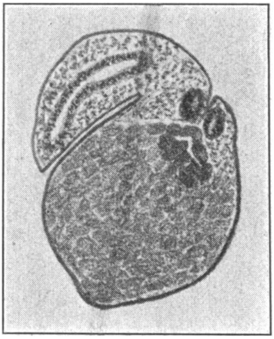

FIG. 16.

section of the cephalic end of the cord is shown. The notochord with the transected enteron and the yolk-cell mass are represented immediately ventral to the tube. In Fig. 15, the cephalic

extremity of the embryo is shown above to the left resting upon the trunk of the embryo. Here again approximately the long axis of the brain tube has been encountered by the section knife. Stratification of brain walls has begun. The enteron together with the notochord lie to the right in the sketch. The mesodermal cells constituting the wall of the pharynx lie immediately ventral to the brain tube. Fig. 16 presents these same general features and, in addition, a bifid appearance of the caudal portion of the neural tube upon the right side of the sketch. The ectoderm folds in between the two tube-halves each of which, while presenting an entire tube, demonstrates its derivation by reason of the thinning of its medial wall. This appearance has been previously referred to in the paper on the condition of spina bifida.

It is to be noted that not one of the histological sections, which are presented, as was mentioned before, as merely typical instances of the appearance of the tissues during the different developmental stages of the embryos, presents any indications whatever either of cell or of tissue disorganization. There is no indication of the presence of degenerated areas of protoplasm either in the yolk mass or in the normal body cavities, nor can there be found any degenerated or extruded nuclei. The organs of the embryo such as the enteron, the brain, the cord, the myomeres, the notochord, and so on, seems to be normally formed and are of the normal size. So far as can be ascertained through the study of longitudinal sections of the myomeres at the level of the bend in the trunk the cause of this phenomenon is not, apparently, referable either to a degeneration or to a falling out of myomeres upon the concave side of the trunk. As might be reasonably expected, however, the myomeres upon this side have a form which departs from the normal in so far as the segments conform to the concavity. The corresponding segments on the opposite side of the fold are consequently relatively elongated. The normal morphological appearances of the tissues argued for a normal physiological activity as well, and this seemed to be attested by the movements and general vitality of the tadpoles. None of these tadpoles was permitted to live, however, to the period of transformation.

In the preceding paper on "The Artificial Production of Spina Bifida" it was noted that, apparently, the proanlagen and anlagen of the embryo were restricted to the pigmented hemisphere of the egg, that the raying of a small localized surface-area of the yolk hemisphere or of the region of the quarter produced invariably the condition of spina bifida in the embryo. It was noted in these embryos, however, that indications of the destruction of cell groups or of definite areas of protoplasm in the adult tadpoles were absent. No gross structural defects of organs aside from the bifid character of the spinal cord were demonstrable. Where the dosage of ultra-violet light ray energy was sufficiently great, however, a mass of protoplasm equal in size to the superficial area affected was extruded from the body of the embryo. This was observed as well in this present experiment. Under such conditions naturally the developed embryo demonstrated both an organic and a structural defect. When the dosage is reduced in amount, however, it is demonstrated both by these and by the spina bifida experiments that the possibility exists for the production of a developmental defect the structural cause of which may not be present or recognizable in the adult. The absence of atypical cytological characters as well as organic defects and of exovates must force us to the conclusion that either such did exist for a longer or shorter period during the development of the embryo and subsequently became incorporated into the structure of the body as the result of an elaborate but delayed chemical transformation, or that there ensued upon the raying a transitory suspension of the physiological activity of the cells affected with a later complete resumption of that activity. Either conclusion must force us to presume, however, the presence of a chemical alteration either of cell content or of cell mass as a direct result of the raying. Indeed, it would be rather difficult to contend that no change was brought about in the chemical composition of the protoplasm in the area affected by the rays.

Since it has been definitely proved that protein substances, carbohydrates and lipins may be altered to some degree in their chemical constitution by this form of energy, it does not appear to be unreasonable to infer that one or all of the corresponding

groups of substances may be susceptible to the influence of the rays while still in the living ovum. Exovation as the direct result of the application of a great amount of energy may be cited as an instance of this alteration. The normal histological features of the embryo, and of the adult tadpoles, however, argue the presence of a process of restoration or of regeneration during the time of development of the embryo of the changes which were brought about through the activity of the rays. It might be assumed, accordingly, that the chemical modification thus artificially produced is gradually rectified during the developmental cycle but during the early stages of this process the substances involved are incapable of entering into their normal functions in the chemical elaboration of the pro-anlagen and anlagen. The resultant action of these altered substances might be interpreted, therefore, as that of an inert body which serves, as was seen to be the case with the spina bifida embryos, as a check to the gross, mechanical shiftings characteristic of the normal genesis of the anlagen. The absence of an area of altered protoplasm or of organic defects in the adult tadpole is the basis for the logical argument that the chemical readjustment of the protoplasm, altered by the rays, is ultimately completed to the degree that this protoplasmic mass does enter finally into its usual function in the elaboration of the normal anlagen.

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